A Taxonomic Review of the Genera *Kitasatosporia* and *Streptovorticillium* by Analysis of Ribosomal Protein AT-L30

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An analysis of the ribosomal AT-L30 proteins from 42 strains of 35 species belonging to the genera *Streptomyces*, *Streptovorticillium*, and *Kitasatosporia* and related genera revealed that all of the members of the genera *Streptovorticillium* and *Kitasatosporia* examined had the same sequence as *Streptomyces exfoliatus* or a highly homologous sequence and exhibited high levels of relatedness to *Streptomyces lavendulae*. These results strongly support the previous suggestion of Witt and Stackebrandt (D. Witt and E. Stackebrandt, *Syst. Appl. Microbiol.* 13:361–371, 1990) and Wellington et al. (E. M. H. Wellington, E. Stackebrandt, D. Sanders, J. Wolstrup, and N. O. G. Jorgensen, *Int. J. Syst. Bacteriol.* 42:156–160, 1992) that the genera *Streptovorticillium* and *Kitasatosporia* should be united with the genus *Streptomyces* on the basis of 16S rRNA data.

Among the actinomycetes, the family *Streptomycetaceae* originally contained the genera *Streptovorticillium*, *Actinopycnidium*, *Microellobosporium*, *Chainia*, *Kitasatoa*, and *Elytrosporangium*. However, all of these genera except the genus *Streptovorticillium* were reclassified by Goodfellow et al. (5–8) because of their phenetic, chemotaxonomic, and genomic similarities (2, 14, 15, 34, 35). The only genus of the family not united with the genus *Streptomyces* was the genus *Streptovorticillium*, probably because the members of this genus have significant morphological characteristics; namely, they form aerial mycelia which consist of long, straight filaments bearing whorls consisting of three to six branches (16). A total of 24 species were included in this genus in *Bergey's Manual of Systematic Bacteriology* (16). The genus *Kitasatosporia* was proposed by Omura et al. (27, 32) in 1982 for newly isolated strains whose cell walls contained similar amounts of the LL and meso isomers of diaminopimelic acid, as well as glycine and galactose; only three species (*Kitasatosporia setae*, *Kitasatosporia griseola*, and *Kitasatosporia phosalacinea*) are listed in this genus in *Bergey's Manual of Systematic Bacteriology* (26). *Kitasatosporia cystarginea* is a recently described species which was originally isolated by Kusakabe and Isono (12). Although the members of the genus *Kitasatosporia* are similar to members of *Streptomyces* spp. in both morphology and physiology, cultivation in liquid media results in production of spore-like cells which contain LL-diaminopimelic acid, whereas the mycelium contains meso-diaminopimelic acid (31). Most *Streptomyces* species do not produce spores in liquid cultures; the only exception is *Streptomyces griseus*, which can produce submerged spores (3). Members of the genus *Kitasatosporia* are also differentiated from *Streptomyces* species by the presence of galactose in whole-cell hydrolysates (27).

The taxonomic status of the genera *Streptovorticillium* and *Kitasatosporia* remained uncertain until members of these taxa were subjected to a phylogenetic analysis. The results of a recent study in which partial 16S rRNA sequences were compared indicated that all of the *Streptomyces* and *Streptovorticillium* strains examined were members of one of several phylogenetically defined lines of descent in the order *Actinomycetales* (36). These organisms did not cluster according to their placement in different genera, and certain members of the genus *Streptomyces* were more closely related to strepto-

vorticillia than to other members of their own genus. On the basis of high levels of phylogenetic similarity, in addition to phenetic similarities (35), Witt and Stackebrandt (36) proposed that the genera *Streptovorticillium* and *Streptomyces* should be combined. Likewise, more recently, Wellington et al. (33) proposed to that the genus *Kitasatosporia* should be synonymous with the genus *Streptomyces* on the basis of phenetic analysis data and on the basis of homology of stretches of 16S rRNA. Nakagaito et al. proposed that *K. cystarginea* should be transferred to the genus *Streptomyces* on the basis of physiological, chemotaxonomic, and DNA-DNA hybridization data (17). (In this paper, for convenience, we use the names *Streptovorticillium* and *Kitasatosporia* instead of the unified name *Streptomyces*.)

Ribosomes and their components have provided an excellent way to study the evolution of organisms, since they are essential components of all cells. During the last few years, we have demonstrated that analysis of ribosomal AT-L30 proteins (homologous to *Escherichia coli* L30 protein) can be used for classification of actinomycetes at the genus level (19–25). The aim of this study was to investigate the taxonomic structure of the streptomycetes.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. Almost all of the strains were type strains that were obtained from the following sources: Japan Collection of Microorganisms, Saitama, Japan; Institute of Fermentation, Yodogawa-ku, Osaka, Japan; Fermentation Research Institute, Tsukuba, Japan; Kitasato Institute, Minato-ku, Tokyo, Japan; Institute of Microbial Chemistry, Shinagawa-ku, Tokyo, Japan; American Type Culture Collection, Rockville, Md; and John Innes Institute, Norwich, England. The strains were grown in soluble starch-Polypeptone-yeast extract medium at 30°C (23).

Preparation of total ribosomal proteins and two-dimensional PAGE. After cultivation for 15 to 20 h (to mid-exponential phase), the cells were collected, washed, and then disrupted by sonication for 3 to 5 min. The 70S ribosomes were pelleted by centrifugation at 110,000 × g for 4 h. Ribosomal proteins were prepared from 70S ribosomes by extraction with acetic acid, as described previously (18), by using the method of Hardy et al. (9). The total ribosomal protein samples obtained in this way contained 20 to 30 mg of protein per ml.

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1	5	10	15	20
1) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
2) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
3) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
4) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
5) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
6) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
7) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
8) Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
9) Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
10) Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
11) Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
12) Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
13) Ala-Gln-Leu-Lys-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu				
14) Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
15) Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
16) Ala-Gln-Leu-Arg-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu				
17) Ala-Gln-Leu-Arg-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu				
18) Ala-Gln-Leu-Arg-Ile-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
19) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu				
20) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu				
21) Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
22) Met-Arg-Ile-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu				
23) Ala-Lys-Ile-Lys-Ile-Thr-Gln-Thr- ? -Ser-Lys-Ile-Gly-Gly-Lys-Gln				
24) Ala-Lys-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Ser-Ile-Gly- ? -Gln-Gln- ? - ? - ? -Asp				
25) Ala- ? -Leu-Lys-Ile-Thr-Gln-Val-Arg-Ser-Lys-Ile-Gly-Gly-Lys-Gln-Asn-Gln- ? -Asp				
26) Ala-Gln-Leu-Lys-Val-Thr-Gln-Val-Lys-Ser-Ser-Ile-Gly-Thr-Lys-Gln-Asn- ? - ? -Asp				
27) Ala-Gln-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ala-Val-Ile-Asn				
28) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Val-Arg-Ser-Lys-Ile-Gly-Gly-Lys-Gln-Asn-Gln- ? -Asp-Ser-Leu- ? - ? -Leu				
29) Ala-Gln-Leu-Lys-Val-Gln-Gln-Lys-Lys-Gly-Leu-Val-Gly-Leu-Lys-Gln-Asn-Gln- ? -Asp				
30) Ala-Arg-Leu-Lys-Val-Thr-Gln-Ile-Arg-Ser-Gly-Ile-Gly- ? -Lys-Gln-Asn-Gln- ? -Asp- ? -Leu				
31) Ala-Glu-Leu-Lys-Ile-Thr-Gln- ? -Lys-Ser-Thr-Ile-Gly-Ala-Lys- ? -Asn-Gln- ? -Asp- ? -Leu				
32) Ala-Arg-Leu-Lys-Val-Thr-Gln-Val-Arg-Ser-Glu-Ile-Gly-Thr-Lys-Lys-Asn-Gln- ? -Asp-Ser-Leu				
33) Ala-Asp-Leu-Lys-Val-Thr-Gln-Ile-Lys-Ser-Thr-Ile-Gly-Ala-Lys-Ala-Asn-Gln-Lys-Asp-Ser-Leu				

FIG. 1. Primary structures of N termini of AT-L30 proteins from various species of the genus *Streptomyces* and related genera. The data for *Actinomadura malachitica* and *Microbispora amethystogenes* are from reference 21, and the data for *Streptosporangium roseum* and *Streptosporangium viridigriseum* subsp. *kofuense* are from reference 22. Question marks indicate amino acids that were not determined. The amino acids that characterize each genus are indicated by boxes. Line 1, *Streptomyces griseus* JCM4644^T (T = type strain); line 2, *Streptomyces griseus* IFO 13189; line 3, *Streptomyces lavendulae* IFO 12789^T; line 4, *Streptomyces lavendulae* MA406-A-1; line 5, *Streptomyces lavendulae* subsp. *grasseri* JCM 4056^T; line 6, *Streptomyces lavendulae* subsp. *fuscus* JCM 5025^T; line 7, *Streptomyces hygroscopicus* IFO 13472^T; line 8, *Streptomyces exfoliatus* JCM 4366^T; line 9, *Streptomyces venezuelae* IFO 13096^T; line 10, *Streptomyces coelicolor* A3(2); line 11, *Streptomyces lividans* 66; line 12, *Streptomyces violaceus* IFO 13103^T; line 13, *Streptomyces parvulus* ATCC 12434^T; line 14, *Streptomyces antibioticus* JCM 4620^T; line 15, *Streptomyces antibioticus* ATCC

TABLE 1. Strains used in this study

Species or subspecies	Strain	Cluster group ^a	Sources ^b
<i>Streptomyces antibioticus</i>	JCM 4620 ^T (= ATCC 23879 ^T) ^c	A31	JCM
<i>Streptomyces antibioticus</i>	ATCC 14888		ATCC
<i>Streptomyces coelicolor</i>	A3(2) ^d		JII
<i>Streptomyces exfoliatus</i>	JCM 4366 ^T (= ATCC 19750 ^T)	A5	JCM
<i>Streptomyces griseoflavus</i>	JCM 4479 ^T (= ATCC 25456 ^T)	A37	JCM
<i>Streptomyces griseoflavus</i>	JCM 4040		JCM
<i>Streptomyces griseoflavus</i>	JCM 4142 (= ATCC 12269)		JCM
<i>Streptomyces griseoflavus</i>	JCM 4878		JCM
<i>Streptomyces griseoflavus</i>	FERM 1805		FERM
<i>Streptomyces griseus</i>	JCM 4644 ^T (= ATCC 23921 ^T)	A1	JCM
<i>Streptomyces griseus</i>	IFO 13189		IFO
<i>Streptomyces hirsutus</i>	JCM 4191 ^T (= ATCC 19773 ^T)	A37	JCM
<i>Streptomyces hygroscopicus</i>	IFO 13472 ^T (= ATCC 27438 ^T)	A32	IFO
<i>Streptomyces lavendulae</i>	IFO 12789 ^T (= ATCC 19777 ^T)	F61	IFO
<i>Streptomyces lavendulae</i>	MA406-A-1		IMC
<i>Streptomyces lavendulae</i> subsp. <i>grassierus</i>	JCM 4056 ^T (= ATCC 15875 ^T)	ND ^e	JCM
<i>Streptomyces lavendulae</i> subsp. <i>fuscus</i>	JCM 5025 ^T	ND	JCM
<i>Streptomyces lividans</i>	66		JII
<i>Streptomyces parvulus</i>	ATCC 12434 ^T	A12	ATCC
<i>Streptomyces prasinus</i>	JCM 4192 ^T (= ATCC 19800 ^T)	A37	JCM
<i>Streptomyces venezuelae</i>	IFO 13096 ^T (= ATCC 25508 ^T)	A6	IFO
<i>Streptomyces violaceus</i>	IFO 13103 ^T (= ATCC 25515 ^T)	A6	IFO
<i>Streptoverticillium baldacii</i> (<i>Streptomyces baldacii</i>) ^f	JCM 4272 ^T (= ATCC 23654 ^T)	ND	JCM
<i>Streptoverticillium cinnamomeum</i> subsp. <i>cinnamomeum</i>	JCM 4152 ^T (= ATCC 23897 ^T)	F55	JCM
(<i>Streptomyces cinnamomeus</i>)			
<i>Streptoverticillium griseocarneum</i> (<i>Streptomyces griseocarneus</i>)	JCM 4095 ^T (= ATCC 19763 ^T)	F55	JCM
<i>Streptoverticillium orinoci</i> (<i>Streptomyces orinoci</i>)	JCM 4807 ^T (= ATCC 23202 ^T)	F58	JCM
<i>Kitasatosporia cystarginea</i> (<i>Streptomyces cystargineus</i>)	JCM 7356 ^T (= ATCC 49931 ^T)		KI
<i>Kitasatosporia griseola</i> (<i>Streptomyces griseolosporus</i>)	KA 341 ^T (= JCM 3339 ^T = DSM 43859 ^T)		KI
<i>Kitasatosporia papulosa</i> (<i>Streptomyces</i> sp.)	KA 655 ^T (= JCM 7250 ^T = ATCC 49231 ^T)		KI
<i>Kitasatosporia phosalacinea</i> (<i>Streptomyces phosalacineus</i>)	KA 338 ^T (= JCM 3340 ^T = DSM 43860 ^T)		KI
<i>Kitasatosporia setae</i> (<i>Streptomyces setae</i>)	JCM 3304 ^T (= ATCC 33774 ^T)		KI
<i>Actinomadura malachitica</i>	JCM 3297 ^T (= ATCC 27888 ^T)		JCM
<i>Actinoplanes phillipinensis</i>	JCM 3001 ^T (= ATCC 12427 ^T)		JCM
<i>Dactylosporangium aurantiacum</i>	IFO 12592 ^T (= ATCC 23491 ^T)		IFO
<i>Microbispora amethystogenes</i>	JCM 3021 ^T		JCM
<i>Micromonospora chalicea</i>	JCM 3031 ^T (= ATCC 12452 ^T)		JCM
<i>Nocardia asteroides</i>	JCM 3384 ^T (= ATCC 19247 ^T)		JCM
<i>Nocardioides flavus</i>	JCM 3334 ^T		JCM
<i>Nocardiopsis dassonvillei</i>	IFO 13908		IFO
<i>Streptosporangium roseum</i>	JCM 3005 ^T (= ATCC 12428 ^T)		JCM
<i>Streptosporangium viridogriseum</i> subsp. <i>kofuense</i>	JCM 3157 ^T (= ATCC 27102 ^T)		JCM
<i>Thermomonospora chromogena</i>	JCM 6244 ^T		JCM

^a Cluster groups of Williams et al. (35).

^b ATCC, American Type Culture Collection; FERM, Fermentation Research Institute; JCM, Japan Collection of Microorganisms; JII, John Innes Institute; IFO, Institute of Fermentation; IMC, Institute of Microbial Chemistry; KI, Kitasato Institute.

^c T = type strain.

^d This strain is a misclassified strain of *Streptomyces violaceoruber*.

^e ND, not determined.

^f The names in parentheses are emended names.

Two-dimensional polyacrylamide gel electrophoresis (PAGE) by the method of Kaltschmidt and Wittmann (10) has been described in detail previously (18).

Determination of amino acid sequence. To prepare AT-L30 protein samples for amino acid sequence analysis, large amounts (7 to 10 mg) of total ribosomal proteins were applied

to PAGE gels. After two-dimensional PAGE, the proteins were extracted from the gels by dry type electrophoresis with Sartoblot II-S (catalog no. SM 17556; Sartorius, Göttingen, Germany), as described below. Electrophoresis was carried out for 6 h at a constant current of 400 mA (initial voltage, 7 V); the protein was extracted from each gel on a membrane

14888; line 16, *Streptomyces hirsutus* JCM 4191^T; line 17, *Streptomyces prasinus* JCM 4192^T; line 18, *Streptomyces griseoflavus* JCM 4479^T; line 19, *Streptomyces griseoflavus* JCM 4040; line 20, *Streptomyces griseoflavus* FERM 1805; line 21, *Streptomyces griseoflavus* JCM 4878; line 22, *Streptomyces griseoflavus* JCM 4142; line 23, *Nocardiopsis dassonvillei* IFO 13908; line 24, *Microbispora amethystogenes* JCM 3021^T; line 25, *Streptosporangium roseum* JCM 3005^T; line 26, *Streptosporangium viridogriseum* subsp. *kofuense* JCM 3157^T; line 27, *Actinomadura malachitica* JCM 3297^T; line 28, *Thermomonospora chromogena* JCM 6244^T; line 29, *Nocardioides flavus* JCM 3334^T; line 30, *Actinoplanes phillipinensis* JCM 3001^T; line 31, *Dactylosporangium aurantiacum* IFO 12592^T; line 32, *Micromonospora chalicea* JCM 3031^T; line 33, *Nocardia asteroides* JCM 3384^T.

TABLE 2. SAS values for the amino acid sequences of AT-L30 proteins^a

Strain	SAS values (%)												
	<i>Streptomyces lavendulae</i> IFO 12789 ^T	<i>Streptomyces exfoliatus</i> JCM 4366 ^T	<i>Streptomyces violaceus</i> IFO 13103 ^T	<i>Streptomyces antibioticus</i> JCM 4620 ^T	<i>Streptomyces griseoflavus</i> JCM 4479 ^T	<i>Microbispora amethystogenes</i> JCM 3021 ^T	<i>Streptosporangium roseum</i> JCM 3005 ^T	<i>Streptosporangium viridogriseum</i> subsp. <i>kofuense</i> JCM 3157 ^T	<i>Thermomonospora chromogena</i> JCM 6244 ^T	<i>Nocardioideus flavus</i> JCM 3334 ^T	<i>Actinoplanes philippinensis</i> JCM 3001 ^T	<i>Dactylosporangium aurantiacum</i> IFO 12592 ^T	<i>Micromonospora chalcone</i> JCM 3031 ^T
<i>Streptomyces exfoliatus</i> JCM 4366 ^T	94												
<i>Streptomyces violaceus</i> IFO 13103 ^T	89	83											
<i>Streptomyces antibioticus</i> JCM 4620 ^T	94	89	94										
<i>Streptomyces griseoflavus</i> JCM 4479 ^T	83	78	94	89									
<i>Microbispora amethystogenes</i> JCM 3021 ^T	81	75	75	81	69								
<i>Streptosporangium roseum</i> JCM 3005 ^T	76	72	82	76	76	73							
<i>Streptosporangium viridogriseum</i> subsp. <i>kofuense</i> JCM 3157 ^T	72	78	83	78	78	75	76						
<i>Thermomonospora chromogena</i> JCM 6244 ^T	78	74	78	72	72	69	100	72					
<i>Nocardioideus flavus</i> JCM 3334 ^T	56	63	61	61	56	50	56	67	53				
<i>Actinoplanes philippinensis</i> JCM 3001 ^T	76	83	71	71	65	63	82	76	83	61			
<i>Dactylosporangium aurantiacum</i> IFO 12592 ^T	81	76	76	81	75	73	81	75	76	59	75		
<i>Micromonospora chalcone</i> JCM 3031 ^T	67	74	67	61	61	56	78	78	79	53	83	71	
<i>Nocardia asteroides</i> JCM 3384 ^T	67	74	67	67	61	63	67	72	63	58	78	88	68

^a The SAS value for each pair of organisms for 20 N-terminal amino acids of the AT-L30 protein was determined on the basis of the data shown in Fig. 1.

(ProBlott; Applied Biosystems, Foster City, Calif.), which was placed on the cathode side. The buffer solution used contained (per liter) 300 g of urea, 14 g of glycine, and 52 ml of acetic acid (adjusted to pH 4.0 with 5 N KOH). After blotting, the membrane was stained with a 0.2% solution of Coomassie blue, and then the spot identified as the AT-L30 protein was cut from the gel. Each spot contained 30 to 100 pmol of protein. The membrane piece with the spot was washed four times with 10 ml of a 60% methanol solution containing 7% acetic acid. The washed piece was used for amino acid sequence analysis. An amino acid sequence analysis of each

AT-L30 protein was performed by using a model 470A protein sequencer (Applied Biosystems). The method which we used was based on the Edman degradation, and determination of a maximum of 25 N-terminal amino acids was possible when about 100 pmol of AT-L30 preparation was used. In *E. coli* the L30 protein that corresponds to actinomycete AT-L30 proteins consists of 58 amino acid residues. The 25 N-terminal amino acids, therefore, represent nearly one-half of the whole AT-L30 protein, assuming that the L30 protein from *E. coli* and the AT-L30 proteins from actinomycetes are the same size.

RESULTS AND DISCUSSION

Amino acid sequences of AT-L30 proteins. (i) *Streptomyces*.

We first analyzed AT-L30 proteins from 22 strains of 12 species belonging to the genus *Streptomyces*, and from strains belonging to other representative actinomycete genera (Fig. 1). [*Streptomyces coelicolor* A3(2) has been shown to be a misclassified strain of *Streptomyces violaceoruber* (1, 13).] Figure 1 shows that we found high levels of amino acid sequence homology within the genus *Streptomyces*, while lower levels of homology were detected among the actinomycete genera. To express quantitatively the levels of similarity of the amino acid sequences (SAS values), we determined the frequency of appearance of the same amino acid in 20 N-terminal amino acids of each AT-L30 protein. The SAS values determined in this way for combinations of representative strains are shown in Table 2. On the basis of the SAS values shown in Table 2, we drew dendrograms (Fig. 2 and 3). The trees were constructed by using a phylogenetic algorithm. The tree in Fig. 2 has three main branches; the first branch includes the genera *Actinoplanes*, *Micromonospora*, *Nocardia*, and *Dactylosporangium*, the second branch includes the genera *Thermomonospora*, *Strep-*

FIG. 2. Clustering based on AT-L30 protein SAS values. The dendrogram was drawn by using the data in Fig. 1.

FIG. 3. Clustering based on AT-L30 protein SAS values. The dendrogram was drawn by using the data in Fig. 1, 4, and 5. S., *Streptomyces*; K., *Kitasatosporia*; Stv., *Streptoverticillium*.

tosporangium, *Microbispora*, and *Streptomyces*, and the third branch contains the genus *Nocardioidea*. The greatest phylogenetic distances were between the genus *Nocardioidea* and the other genera. In general, our classification was very similar to the classification of Stackebrandt and his colleagues (11, 28–30), which was based on 16S rRNA data. The results of Stackebrandt et al. are summarized and shown in a dendrogram in *Bergey's Manual of Systematic Bacteriology* (4). The only difference was that the genera *Thermomonospora* and *Streptosporangium* appeared to be closely related on the basis of our data, whereas those taxa were rather distantly related as determined by the 16S rRNA analysis (4).

The members of the genus *Streptomyces* examined exhibited high levels of homology (SAS values, 83 to 100%) within the genus, and three main branches were identified (Fig. 3). The first branch included *Streptomyces lavendulae*, *Streptomyces griseus*, *Streptomyces hygroscopicus*, *Streptomyces exfoliatus*, and *Streptomyces venezuelae*. The second branch included *Streptomyces violaceus*, *Streptomyces parvulus*, *Streptomyces coelicolor* A3(2), *Streptomyces lividans* 66, and *Streptomyces antibioticus*. The third cluster, whose members appeared to be distantly related to the other taxa, included *Streptomyces griseoflavus*, *Streptomyces hirsutus*, and *Streptomyces prasinus*; the members of this cluster were characterized by arginine at position 4 (Fig. 1). There were many combinations which produced an SAS value of 100% and thus formed subclusters on the phylogenetic tree. As expected, in most cases strains classified in the same species exhibited 100% homology in their amino acid sequences; this was true for strains of *Streptomyces lavendulae*,

Streptomyces griseus, and *Streptomyces antibioticus* (Fig. 1 and 3). The exceptions were strains assigned to *Streptomyces griseoflavus*; four of the strains examined (JCM 4040, JCM 4142, JCM 4878, and FERM 1805) were classified in taxa different from the taxon that contained the type strain of *Streptomyces griseoflavus*, strain JCM 4479. In the light of the findings described above, these four strains were apparently misidentified as *Streptomyces griseoflavus*, unless variability in the amino acid sequence of the AT-L30 protein is a characteristic typical of this species. In particular, the N-terminal amino acid sequence (Met-Arg-Ile) found in strain JCM 4142 was peculiar since this sequence has not been found in the N terminus of any other actinomycete AT-L30 protein examined so far. This fact may imply that strain JCM 4142 should be classified in a new species or in a species which has not been subjected to AT-L30 analyses.

(ii) *Streptoverticillium*. Members of four species classified in the genus *Streptoverticillium*, including *Streptoverticillium baldaccii* JCM 4272 (the type strain of the type species), were subjected to an amino acid sequence analysis (Fig. 4). The four species exhibited high levels of homology (SAS values, 94 to 100%) in comparisons with each other; 100% homology was detected among *Streptoverticillium baldaccii*, *Streptoverticillium cinnamoneum* subsp. *cinnamoneum*, and *Streptoverticillium griseocarneum* (Fig. 3). These organisms were most closely related to *Streptomyces exfoliatus* and were relatively closely related to *Streptomyces lavendulae*, but they were distantly related to *Streptomyces violaceus*, *Streptomyces coelicolor* A3(2), and *Streptomyces antibioticus*. *Streptoverticillium orinoci*

1	5	10	15	20
1) Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
2) Ala-Arg-Leu-Lys-Val-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
3) Ala-Arg-Leu-Lys-Val-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
4) Ala-Arg-Leu-Lys-Val-Thr-Gln-Val-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
5) Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu- ? -Ser-Leu				

FIG. 4. Primary structures of N termini of AT-L30 proteins from several species of the genus *Streptovorticillium*. The amino acids that characterized the genus *Streptomyces* are boxed. Line 1, *Streptomyces exfoliatus* JCM 4366^T; line 2, *Streptovorticillium baldaccii* JCM 4272^T; line 3, *Streptovorticillium griseocarneum* JCM 4095^T; line 4, *Streptovorticillium cinnamoneum* subsp. *cinnamoneum* JCM 4152^T; line 5, *Streptovorticillium orinoci* JCM 4807^T.

exhibited 100% homology to *Streptomyces exfoliatus*. These results agree with the 16S rRNA analysis results of Witt and Stackebrandt (36) and thus strongly support the suggestion of these authors that the genera *Streptovorticillium* and *Streptomyces* should be united.

(iii) *Kitasatosporia*. Members of five species classified in the genus *Kitasatosporia*, including *K. setae* JCM 3304 (the type strain of the type species), exhibited high levels of homology (SAS values, 94 to 100%) in comparisons with each other (Fig. 5); 100% homology was detected among *K. setae*, *Kitasatosporia papulosa*, *K. phosalacinea*, and *K. griseola*. These four species exhibited 100% homology to *Streptomyces exfoliatus* and were closely related to *Streptomyces lavendulae* and streptovorticillia (SAS value, 94%) (Fig. 3); similar conclusions were obtained from a 16S rRNA analysis (33). *K. cystarginea* exhibited 100% homology to *Streptomyces lavendulae*. These results clearly indicate that the members of the genus *Kitasatosporia* should be placed in the genus *Streptomyces*, as suggested by Wellington et al. (33).

Amino acid sequences characteristic of the genus *Streptomyces*. In addition to simple comparisons of the levels of homology of amino acid sequences, it was possible to pinpoint amino acids located at specific positions which characterize each genus. This has been done elsewhere for the genera *Escherichia*, *Bacillus*, *Staphylococcus*, *Thermoactinomyces*, and *Thermomonospora* (20a). The amino acid located at position 12 (position 11 in actinomycetes) was a particularly important signature amino acid for classification at the genus level. Furthermore, in the genus *Streptomyces*, position 11 was characterized by tyrosine (Fig. 1), which is an amino acid that has not been found in the other AT-L30 proteins examined so far. In other genera of actinomycetes, position 11 was occupied by lysine, serine, valine, leucine, glycine, threonine, or glutamic acid (Fig. 1). Thus, the occupation of position 11 by tyrosine appears to be a characteristic typical of the genus *Streptomyces*.

Also, serine at position 14 and threonine at position 21 are characteristic of this genus. It should also be noted that the strains classified in the genera *Streptovorticillium* and *Kitasatosporia* all had tyrosine, serine, and threonine at positions 11, 14, and 21, respectively (Fig. 4 and 5). Thus, the suggestion that these genera should be combined with the genus *Streptomyces* was supported by the amino acids that characterize each genus.

In this study, using our new approach, we confirmed the previous conclusions of Witt and Stackebrandt (36) and Wellington et al. (33), which were based on 16S rRNA analysis data. Thus, we reached the same conclusion that the genera *Streptovorticillium* and *Kitasatosporia* should be united with the genus *Streptomyces*. The agreement of phylogenetic relatedness data for various actinomycete genera obtained when our method (Fig. 2) and the 16S rRNA analysis method (4) were used is also notable. These facts, together with previous results (18–25), verify the efficacy of ribosomal protein AT-L30 analysis for identification and classification of actinomycetes.

Williams et al. (35) described phenetic classification of *Streptomyces* species; in their study, 394 type cultures of *Streptomyces* species were subjected to a numerical classification analysis based on 139 criteria. The findings of Williams et al. were consistent with our data in several respects. (i) *Streptomyces hirsutus* and *Streptomyces prasinus*, both of which were placed in *Streptomyces griseoflavus* cluster A37 of Williams et al., exhibited 100% sequence homology to the type strain of *Streptomyces griseoflavus*. (ii) *Streptovorticillium cinnamoneum* and *Streptovorticillium griseocarneum*, both of which were placed in *Streptomyces* cluster F55, exhibited 100% sequence homology to one another. (iii) Streptovorticillia (*Streptovorticillium cinnamoneum*, *Streptovorticillium griseocarneum*, *Streptovorticillium baldaccii*, and *Streptovorticillium orinoci*) which were classified in cluster F along with *Streptomyces lavendulae* all exhibited high levels of homology to *Streptomyces lavendulae*. However, a difference between the two studies

1	5	10	15	20
1) Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
2) Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
3) Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				
4) Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
5) Ala-Arg-Leu-Lys-Val-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp				
6) Ala-Arg-Leu-Lys-Ile-Thr-Gln-Thr-Lys-Ser-Tyr-Ile-Gly-Ser-Lys-Gln-Asn- ? - ? -Asp-Thr-Leu				

FIG. 5. Primary structures of N termini of AT-L30 proteins from several species of the genus *Kitasatosporia*. The amino acids that characterized the genus *Streptomyces* are boxed. Line 1, *Streptomyces exfoliatus* JCM 4366^T; line 2, *K. setae* JCM 3304^T; line 3, *K. griseola* KA 341^T; line 4, *K. phosalacinea* KA 338^T; line 5, *K. papulosa* KA 655^T; line 6, *K. cystarginea* JCM 7356^T.

was also found; although *Streptomyces violaceus* and *Streptomyces venezuelae* were both classified in cluster A6 of Williams et al., these species were placed in different clusters on the basis of our AT-L30 sequence analysis data. Drawing conclusions concerning species relationships might lead to overinterpretation of the data when highly conserved molecules, such as ribosomal proteins, are involved.

Streptomyces lavendulae occupies a rather distant taxonomic position (cluster group F) compared with other *Streptomyces* species in numerical classification systems (35). The 16S rRNA data also indicated that *Streptomyces lavendulae* should be separated from other streptomycetes and belongs in a subgroup that contains strains previously classified as members of the genus *Streptoverticillium*, indicating that *Streptomyces lavendulae* is more closely related to verticillium-bearing strains than to strains that produce straight or spiral chains (36). Despite the agreement of the phenetic and 16S rRNA data, our study revealed that *Streptomyces lavendulae* is more closely related to the members of cluster group A, such as *Streptomyces griseus*, than to streptoverticillia. No explanation can be offered at this time, but it does seem likely that in this case the true relationships are those that are based on 16S rRNA analysis data, given the excellent agreement with the phenetic data. More work on *Streptomyces* AT-L30 analysis is clearly needed.

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